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1 **Title: Durability of SARS-CoV-2-specific T cell responses at 12-months post-infection.**

2 **Running head: SARS-CoV-2-specific T cells are detectable at 12 months**

3

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45 **Abstract**

46 **Background**. Characterizing the longevity and quality of cellular immune responses to SARS-
47 CoV-2 is critical to understanding immunologic approaches to protection against COVID-19. Prior
48 studies suggest SARS-CoV-2-specific T cells are present in peripheral blood 10 months after
49 infection. Further analysis of the function, durability, and diversity of the cellular response long
50 after natural infection, over a wider range of ages and disease phenotypes, is needed to further
51 identify preventative and therapeutic interventions.

52 **Methods**. We identified participants in our multi-site longitudinal, prospective cohort study 12-
53 months post SARS-CoV-2 infection representing a range of disease severity. We investigated the
54 function, phenotypes, and frequency of T cells specific for SARS-CoV-2 using intracellular
55 cytokine staining and spectral flow cytometry. In parallel, the magnitude of SARS-CoV-2-specific
56 antibodies was compared.

57 **Results**. SARS-CoV-2-specific antibodies and T cells were detected at 12-months post-infection.
58 Severity of acute illness was associated with higher frequencies of SARS-CoV-2-specific CD4 T
59 cells and antibodies at 12-months. In contrast, polyfunctional and cytotoxic T cells responsive to
60 SARS-CoV-2 were identified in participants over a wide spectrum of disease severity.

61 **Conclusions**. Our data show that SARS-CoV-2 infection induces polyfunctional memory T cells
62 detectable at 12-months post-infection, with higher frequency noted in those who originally
63 experienced severe disease.

64

65 **Key words: COVID-19, SARS-CoV-2, 12-months, T cell, antibody, memory, cytotoxicity,**
66 **polyfunctionality, durability**

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67 **Introduction**

68 Understanding the development and durability of protective immune responses against SARS-
69 CoV-2 remains critical as we seek global reduction in disease burden. Antibody responses induced
70 during a primary SARS-CoV-2 infection have been shown to wane, but may be present in the
71 circulation up to twelve months post symptoms onset (PSO) [1, 2]. Thus far, SARS-CoV-2-
72 specific T cells have been detected up to 10 months PSO [3, 4]. Intriguingly, SARS-CoV-1-
73 specific T cells have been identified 17 years post-infection, suggesting the potential for very long-
74 lived T cell memory [5]. Antibodies have been effective in reducing the disease burden of SARS-
75 CoV-2, however, infection still occurs requiring the recruitment of T cells to clear infected cells.
76 In order to utilize the specificity and potent viral clearance of T cells, further knowledge is required
77 regarding viral antigen specificity, memory differentiation and longevity [6].

78 Studies on SARS-CoV-2-specific T cells within two months PSO suggests that potent
79 antigen-specific T cells are associated with mild disease, whereas a lack of these antiviral cells or
80 a delay in development is associated with severe disease [7, 8]. Peng et al. [9] showed that
81 individuals who had mild disease had a higher ratio of polyfunctional CD8 T cells compared to
82 CD4 T cells around 42 days PSO, suggesting that potent SARS-CoV-2-specific CD8 T cells may
83 be protective. Analysis of functional T cell responses at memory time points is needed to provide
84 insight into their cytolytic potential and role in protection upon re-infection that prior studies
85 relying on activation-induced markers (AIM) [6] could not.

86 The T cell response to SARS-CoV-2 has been shown to recognize epitopes across multiple
87 viral proteins including the spike glycoprotein (S), nucleocapsid (N), membrane (M), and small
88 envelope (E) proteins, as well as other nonstructural proteins (nsp) [10, 11]. Due to the ability of
89 T cells to recognize structural and nonstructural proteins that are less susceptible to antibody-

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90 dependent mutational pressure, T cells may provide cross-reactive protection against other
91 coronaviruses as well as SARS-CoV-2 variants [12, 13].
92 In this study, we analyzed the durability and functional characteristics of the SARS-CoV-
93 2-specific memory T cell response at 12-months PSO derived from a prospective, longitudinal
94 cohort of United States Military Health System (MHS) beneficiaries, including active-duty
95 military and dependents with varying severity of disease. Understanding the durability and
96 functional response of SARS-CoV-2-specific T cells can help determine how the humoral and
97 cellular components of antiviral immunity work synergistically to prevent future infection and
98 inform vaccine strategies.
99
100 **Methods and Materials:**
101 **Study Participants**
102 Individuals were enrolled into the Epidemiology, Immunology, and Clinical Characteristics of
103 Emerging Infectious Diseases with Pandemic Potential (EPICC) study, a prospective, longitudinal
104 cohort study, if they were exposed to, had symptoms consistent with, or had documented SARS-
105 CoV-2 infection beginning in March of 2020. Samples from four of ten military treatment facilities
106 (MTFs), which included Walter Reed National Military Medical Center (Bethesda, MD), Brooke
107 Army Medical Center (San Antonio, TX), Naval Medical Center San Diego (San Diego, CA), and
108 Madigan Army Medical Center (Tacoma, WA) were included based on peripheral blood
109 mononuclear cells (PBMCs) obtained 12-months PSO and absence of evidence for re-infection.
110 Infection was confirmed by reverse-transcriptase polymerase chain reaction (RT-PCR) and
111 serologic response. Study participants included in this analysis were followed for 12-months PSO
112 with biological samples and clinical questionnaire data collected throughout the duration of the

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113 study. Receipt of a SARS-CoV-2 vaccine was identified by report and confirmed by medical data
114 repository review. Study participants who received a SARS-CoV-2 vaccine prior to the 12-month
115 peripheral blood collection were excluded from S-specific T cell and antibody analysis. Study
116 participants were evaluated for re-infection by documented PCR positive for SARS-CoV-2 or a
117 significant rise in both S and N-specific antibody responses. Participants who were re-infected
118 were excluded for comparative T cell and antibody analysis in the current study. The protocol was
119 approved by the Uniformed Services University Institutional Review Board (IDCRP-085), and all
120 subjects or their legally authorized representative provided informed consent to participate.
121
122 **PBMC and serum preparation**
123 PBMCs were isolated from peripheral whole blood collected in acid citrate dextrose (ACD) tubes
124 at 12-months PSO. PBMCs were purified using a Ficoll-Histopaque (Fisher Scientific, NH)
125 gradient. Cells were preserved in 90% fetal bovine serum (Sigma-Aldrich, MO) and 10% dimethyl
126 sulfoxide (DMSO, Sigma-Aldrich, MO) and stored in liquid nitrogen. Serum was isolated from
127 blood collection in EDTA tubes.
128
129 **Measurement of antigen presenting cells**
130 One million thawed PBMCs were stained in PBS at 4°C for 20 minutes for antigen presenting cells
131 (APCs) using the antibodies listed in Supplemental Table 1 and were acquired on a CYTEK Aurora
132 5-laser spectral flow cytometer (CYTEK Biosciences, CA).
133
134 **Antigen-specific T cell analysis**

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135 For identification of antigen-specific T cells, isolated PBMCs were cultured in 96-well plates and
136 stimulated overnight with peptide pools derived from selected viral proteins. Peptide pools were
137 comprised of 15-mer peptides overlapping by 11 amino acid residues covering the S, M, N, and E
138 proteins of SARS-CoV-2 (JPT, Germany), the S protein of endemic human coronavirus (hCoV)
139 strains: HKU1, 229E, NL63 and OC43; and a CMV peptide pool including pp50, pp65, IE1, IE2
140 and envelope glycoprotein B (Mabtech, OH) at a final concentration of 1 mg/ml for each individual
141 peptide (Supplemental Table 2). Monensin (BD Biosciences, CA; BioLegend, CA) was added to
142 the wells 1 hour after peptide addition to prevent cytokine secretion as recommended by the
143 manufacturer. Ten minutes after addition of the peptide pools, CD107a antibody (BioLegend, CA)
144 was added. Cell surface markers were identified with the antibodies listed in Supplemental Table
145 3. Surface staining was followed by cell permeabilization using FoxP3/transcription factor staining
146 buffer set (Thermo Fisher Scientific, MA) at 4°C for at least 30 minutes. Antibodies used for
147 intracellular cytokine staining (ICS) (Supplemental Table 3) were then added. PBMCs cultured in
148 medium without peptide stimulation served as the negative control, or with CytoStim (Miltenyi
149 Biotec, CA) as the positive control for the assay. Samples were acquired on a CYTEK Aurora
150 (CYTEK Biosciences, CA). All cytometric data were analyzed using FlowJo software (BD
151 Biosciences, CA). Samples were considered positive if the frequency of IFNγ+ T cells was 2-fold
152 higher than the medium control and greater than 0.01% of CD4 or CD8 T cells after subtracting
153 the medium control value [14].
154

155 **Multiplex microsphere-based immunoassay screening procedures**

156 Detailed experimental procedures of SARS-CoV-2 S and N protein-based multiplex microsphere
157 immunoassays have been previously described [2, 15]. Briefly, diluted serum and capillary blood

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158 samples were added to 96-well microtiter plates containing antigen-coupled microspheres and
159 tested in technical duplicates. After 45 minutes of agitation, wells were washed, and biotin-
160 conjugated goat anti-human IgG (Thermo Fisher Scientific, Waltham, MA) diluted in PBS +
161 0.05% Tween20 (PBST) was added to each well. Wells were subsequently washed again, then
162 streptavidin-phycoerythrin (1:1000 in PBST) (Bio-Rad, Hercules, CA) was added to each well. A
163 final wash was performed after 45 minutes of incubation, and antigen-antibody complexes were
164 analyzed on BioPlex 200 multiplexing systems (Bio-Rad) for IgG binding, and median
165 fluorescence intensity (MFI) values were reported for specificity to SARS-CoV-2 S and N protein.
166 Participants were excluded from 12-months PSO evaluation based on observed increases in SARS-
167 CoV-2 S protein binding from 6-12 months collections across longitudinal serum samples and an
168 accompanying rise in N protein IgG results, suggesting possible re-infections.
169

170 **Statistical analysis**

171 Data were analyzed using GraphPad Prism 9 (GraphPad Software Inc, CA). Two-group test
172 significance levels were calculated using Mann-Whitney analysis. Correlation coefficients and
173 significance levels were calculated using Spearman rank correlation. A p-value $< 0.05$ was
174 considered statistically significant.
175

176 **Results**

177 **Study cohort**

178 From our cohort, we identified 29 patients who had COVID-19 approximately 1 year prior, and
179 did not have evidence of re-infection prior to a peripheral blood collection at 12-months PSO.
180 Disease severity was classified by inpatient or outpatient status during the acute phase of illness.

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181 The age range of the study participants was 20-72 years of age and the overall distribution of age
182 did not vary significantly (p = 0.1) between inpatients (median age 50.6 with a range of 21.4 to
183 72.4) and outpatients (median age 44.6 with a range of 20.1 to 60.1), nor did sex (Table 1,
184 Supplemental Table 4). Inpatients, however, were more likely to have a comorbidity with
185 respiratory and pulmonary illnesses being the most common. 6/14 study inpatients received
186 intermittent intranasal or inhaled corticosteroids for underlying conditions, in contrast to 1/15
187 outpatient. Two study participant received systemic steroids during acute illness (Table 1,
188 Supplemental Table 4). Data on S-specific humoral and cellular responses was not included for
189 individuals receiving vaccination prior to the 12-month blood drawn. Patients hospitalized during
190 acute illness were further dichotomized into those who required supplemental oxygen during
191 hospitalization and those who did not. Further demographic and clinical information are provided
192 in Supplemental Table 4.
193
194 **Convalescent T cell and antibody responses are detectable 12-months post symptoms onset**
195 Identification of SARS-CoV-2-specific T cells was performed by stimulating PBMCs with peptide
196 pools derived from the S, N, M, and E proteins. Stimulation of PBMCs with these peptide pools
197 demonstrated the presence of SARS-CoV-2-specific CD4 and CD8 T cells at 12-months PSO by
198 ICS (Figure 1A; Supplemental Figure 1A) in 22/29 (75.9%) of study participants. Only three of
199 29 participants had a measurable response to E (data not shown); therefore, the presented data
200 focused on S, M, and N. Stimulation with the CMV peptide pool demonstrated CMV-specific CD4
201 and CD8 T cell responses in 73.9% of study participants. Overall, SARS-CoV-2-specific CD4 T
202 cells were more frequently identified in the peripheral blood compared to SARS-CoV-2-specific
203 CD8 T cells. In addition, T cell responses to endemic hCoV strains; HKU1, 229E, OC43, and

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204 NL63 were sporadic, with no differential distribution or magnitude difference between inpatients
205 and outpatients identified (Table 2).

206 The SARS-CoV-2-specific antibody response against S and N proteins were also present
207 in both unvaccinated inpatient and outpatient groups at 12-months PSO (Figure 1B, Supplemental
208 Figure 1B). We found that the antibody response against N in inpatients was higher than in
209 outpatients, in whom it waned to levels below the threshold of endemic hCoV N responses (P =
210 0.01). These findings demonstrate that the humoral and cellular responses to SARS-CoV-2 exhibit
211 durability at 12-months PSO, but vary by severity of disease and antigen specificity.

212 Stratification of study participants by inpatient and outpatient status at peak disease
213 severity demonstrated that individuals with more severe disease exhibited the highest frequency
214 of CD4 T cells responsive to N (P = 0.02), M (P = 0.002), and S (P = 0.064) proteins, regardless
215 of oxygen requirement (Figure 1C). Of the inpatients, 11 of 13 (84.6%) exhibited T cell responses
216 specific for either N or M (P30 and P41 did not exhibit T cell responses above assay cutoff). In
217 contrast, 7 of 14 (50.0%) outpatients exhibited CD4 T cell responses to N or M. Individuals who
218 exhibited T cell specificity for either N or M at 12-months were also more likely (P = 0.0007) to
219 simultaneously recognize other SARS-CoV-2 epitopes in N or M (Supplemental Figure 1C).
220 Compared with the CD4 T cell response, the CD8 T cell response, as measured by IFNγ
221 production, was lower in magnitude and did not correlate with severity of disease (Figure 2D).

222 Quantification of the APCs and frequency of total T cells at 12-months PSO were measured
223 by flow cytometry (Supplemental Figure 1D) to determine whether differences exhibited between
224 study groups were influenced by APC or T cell availability. Our data show that the ratio of total
225 CD4 and CD8 T cells (Supplemental Figure 1E), and the frequency of classical APCs, including
226 conventional dendritic cells 1 and 2 (cDC1/2), plasmacytoid DCs (pDCs) and monocytes

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227 (Supplemental Figure 1F), were similar among patient groups at 12-months PSO and healthy
228 controls.
229

230 **SARS-CoV-2-specific T cells at 12-months post symptoms onset predominantly exhibit**
231 **central memory characteristics**

232 SARS-CoV-2-specific T cells identified by IFNγ production in response to SARS-CoV-2 peptides
233 were characterized with established phenotypic markers of differentiation as previously described
234 [6]. Expression of the lymph node homing receptor, CCR7, coupled with the absence of CD45RA
235 expression is characteristic of central memory T cells [16] (Figure 2A), which are also
236 CD27+CD28+ [17]. This was the predominant phenotype exhibited by CD4 T cells responding to
237 N (Figure 2B; Supplemental Figure 2A) and was distinct from the predominance of naïve T cells
238 in the total CD4 T cell population. The phenotypic distribution of CD4 T cells responding to M
239 and S was similarly skewed towards a central memory phenotype (Supplemental Figure 2B). In
240 contrast, CD8 T cells specific for N and S derived from multiple compartments, including naïve
241 (CCR7+CD45RA+), effector memory T cells re-expressing CD45RA (TEMRA), central and
242 effector memory phenotypes (Figure 2C; Supplemental Figure 2C).

243 Together these findings demonstrate that the SARS-CoV-2-specific T cell responses
244 exhibit a longevity of at least 12-months, with a predominance of central memory CD4 T cells
245 specific for S, N and M proteins. SARS-CoV-2-specific CD8 T cells were less frequent in the
246 peripheral blood and exhibited more diverse memory phenotypes. For both CD4 and CD8 T cells,
247 the severity of disease during acute illness was not reflected in distinct memory phenotype
248 differentiation at 12-months PSO.
249

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250 **Cytokine profiles indicate that SARS-CoV-2-specific memory T cells are polyfunctional**

251 T cells were evaluated for the expression of multiple cytokines in response to the distinct SARS-
252 CoV-2 peptide pools to determine their functional potential. CD4 T cells responsive to N exhibited
253 expression of both IFNγ and IL2, indicating a polyfunctional response in all study participants in
254 both inpatient and outpatient severity groups at 12-months PSO (Figure 3A). Overall, inpatients
255 had a higher frequency of cytokine-expressing CD4 T cells, but the frequency of polyfunctional
256 CD4 T cells did not differ significantly between severity groups (Figure 3A). CD4 T cell responses
257 against M and S exhibited similar frequencies of polyfunctional T cells, suggesting that epitope
258 specificity did not predict polyfunctionality (Supplementary Figure 3A-B). At 12-months, we did
259 not detect expression of IL17A, IL21, IL4 or IL13 by CD4 T cells in response to the evaluated
260 peptide pools (data not shown) based on the assay cut-off, suggesting that type 1 helper (Th1) CD4
261 T cells were dominant over Th2, Th17, or circulating T follicular helper cells (cTfh).

262 CD8 T cells expressing IFNγ were further evaluated for cytolytic potential by the
263 expression of Granzyme B and surface exposure of the lysosome-associated membrane protein
264 CD107a, a marker of degranulation (Figure 3D). The CD8 T cell response to N and S displayed
265 heterogeneity in cytolytic potential, with cells generally expressing either marker in response to
266 their cognate antigens (Figure 3B; Supplemental Figure 3B). Again, we saw no difference in
267 frequency and function of the SARS-CoV-2-specific CD8 T cells based on disease severity.

268 We further looked into the activation features of the IFNγ+ CD4 T cells. Upon peptide
269 stimulation, SARS-CoV-2-specific IFNγ+ CD4 T cells upregulated CD69 and PD-1 compared to
270 the total CD4 T cell population, and was similar in inpatients and outpatients (Figure 3C;
271 Supplementary Figure 3C-D). The other activation markers, including CD38, HLA-DR, or CTLA-

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4, in the IFNγ+ T cells are comparable to the total CD4 T cell population (Supplementary Figure 3C).

Together these data suggest that the memory CD4 T cell response against SARS-CoV-2 predominantly exhibits a Th1 type profile, and that SARS-CoV-2-specific memory CD8 T cells display cytolytic potential at 12-months.

**Discussion**

Here we show that SARS-CoV-2-specific T cell responses are detected at 12-months PSO, extended our prior understanding of the durability of the T cell response against SARS-CoV-2 [4]. The frequency of SARS-CoV-2-specific CD4, but not CD8, T cells was higher at 12-months PSO in individuals who experienced severe disease compared to those who had mild disease at the acute phase. Importantly, memory SARS-CoV-2-specific CD4 T and CD8 T cells exhibited polyfunctionality and cytotoxicity, respectively, suggesting strong recall responses. Our findings and that of others also show that antibodies to SARS-CoV-2 persist 12-months PSO, but wane rapidly [1, 2, 18] and in the case of N-specific antibodies may be undetectable.

Overarchingly, we identified SARS-CoV-2-specific T cell responses in 75.9% of study participants at 12-months PSO using peptide pools derived from SARS-CoV-2 structural proteins N, M, E, and S. Other studies have shown that epitopes in SARS-CoV-2 N, M, and S, together with nsp3, 4, ORF3a are recognized by CD4 and CD8 T cells representing the majority of T cell responses at convalescence, while SARS-CoV-2 E was less frequently recognized [11, 19, 20]. Although we did not map T cell epitopes, we overall observed the same trend of T cell responses to corresponding peptide pools used in this study, but at a much later time point. As the magnitude of CD8 T cell responses contracts within a month of disease onset in humans vaccinated with live

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295 yellow fever virus and smallpox vaccines [21, 22], similarly in COVID-19 patients [23], SARS-
296 CoV-2-specific CD8 T cells in the peripheral blood are of low frequency at one year PSO.
297 Importantly, T cell recognition of multiple epitopes was common within study participants,
298 suggesting broad epitope recognition.

299 Study participants who were hospitalized during acute infection demonstrated the highest
300 frequency of SARS-CoV-2-specific CD4 T cell responses and antibodies to SARS-CoV-2 N. Why
301 severe COVID-19 is associated with a higher memory response for CD4 T cells remains unclear.
302 Since severe disease is associated with higher viral burden [24-26], one hypothesis is that higher
303 viral load at acute infection increases stimulation and antigen availability for T cells expanding
304 more durable responses. However, due to differences in timing of nasal swab acquisition and the
305 size of this subcohort, we were unable to correlate T cell longevity and viral load at acute infection.
306 Published data have yet to show that initial SARS-CoV-2 viral load correlates to the longevity of
307 cellular immunity. Indeed, the longevity of memory T cells is affected by multiple factors
308 including the cytokines such as IL7 and IL15 (reviewed in [27]) as well as the density of antigens
309 presented by APCs, including B cells [28].

310 Importantly, our data show that SARS-CoV-2-specific CD4 T cells present in individuals
311 infected 12-months prior exhibit differentiation toward central memory phenotypes, activation by
312 expression of CD69 and PD-1, and polyfunctionality by expression of IFNγ and IL2. In other viral
313 infections such as influenza, polyfunctional CD4 T cells expressing IFNγ, IL2, and TNFα, are
314 more frequent in convalescent patients who better controlled infection [29]. COVID-19 patients
315 with milder symptoms also had more polyfunctional T cells expressing IFNγ, IL2, and TNFα [30]
316 while severe and critical patients tended to have restricted functional T cells 1-2 months PSO [31].
317 CD4 T cells 1-2 months after SARS-CoV-2 infection also upregulated CD69, CD38, HLA-II,

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318 CTLA-4, and PD-1, as an indication of activation [31, 32]. While the polyfunctionality and
319 activation of long-term memory CD4 T cells is less well characterized in other viral infections and
320 could be impacted by factors such as age and antigen concentration [33-35], in our study, the
321 SARS-CoV-2-specific CD4 T cells between severity study groups were similarly polyfunctional
322 and equally expressed CD69 and PD-1 at 12-months PSO. In addition, although CD8 T cells were
323 less frequent compared to CD4 T cells at 12-months PSO, SARS-CoV-2-specific CD8 T cells
324 exhibited cytotoxicity while CD4 T cells did not (data not shown). Memory CD4 T cells are,
325 however, important in promoting CD8 T cell cytotoxicity and viral clearance (reviewed in [36]).

326 SARS-CoV-2 shares epitopes with endemic hCoVs including HKU1, 229E, NL63, and
327 OC43 [32, 37]. Our analysis was restricted to hCoV peptide pools comprised of S from these
328 viruses and we detected low frequencies of T cell recognition of endemic hCoV that did not differ
329 between patient severity groups. Analysis of additional structural and non-structural protein-
330 derived peptides in larger study cohorts may yield more information regarding the correlation of
331 cross-reactive T cell responses. Moreover, T cells can provide ostensibly broad coverage over
332 SARS-CoV-2 variants of concern, whereas antibodies primarily target S [13]. Data has shown that
333 associated mutations can develop and emerge in the receptor binding domain and other sites of the
334 S glycoprotein that limit prior antibody binding [38-40]. However, due to the unique requirements
335 of peptide presentation in the context of an HLA molecule, distinct epitope-HLA haplotype
336 interactions between individuals present an overwhelming number of mutational target sites for
337 which the virus is unable to evade.

338 Limitations to our study include the challenge of obtaining biological samples at 12-months
339 PSO prior to vaccination in our MHS cohort, which had timely and reliable access to vaccines.
340 Fifty percent of individuals in our cohort were vaccinated prior to a 12-month draw (Table 1). For

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341 these individuals we did not include their S-specific T cell or antibody responses, but did include
342 their N, M and E humoral or cellular responses since these are not components of the mRNA-
343 based vaccines (the vaccines primarily available to our study population). A strength of our cohort
344 is that study participants are followed within the MHS and their epidemiologic, clinical and
345 COVID-19 testing records, and vaccination status are maintained primarily from electronic health
346 record. In addition, sera were analyzed longitudinally to avoid re-infected participants for 12-
347 months T cell analysis.

348 In summary, our data show that both SARS-CoV-2 humoral and cellular responses are
349 measurable 12-months PSO in individuals across a spectrum of disease phenotypes. The
350 magnitude of the CD4 T cell and antibody responses more closely correlated with acute disease
351 severity. Importantly, the memory phenotype and polyfunctional response of the SARS-CoV-2
352 specific T cells did not differ by disease severity. The breadth of T cell epitope recognition by
353 these memory T cells may provide more durable protection against emerging SARS-CoV-2
354 variants.

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436

437 **Figure legends**

438 **Figure 1. Adaptive immune responses to SARS-CoV-2 viral components at 12-months post**
439 **symptoms onset. A.** SARS-CoV-2-specific CD4 and CD8 T cell responses were detected at 12-
440 months PSO by expression of IFNγ upon peptide stimulation and intracellular cytokine staining.
441 **B.** Antibodies to SARS-CoV-2 nucleocapsid protein at 12-months post-infection. Threshold shows
442 the global MFI of antibodies to endemic hCoVs using the same sera in the same assay. **C**.
443 Frequency of CD4 T cell responses to the tested viral components N, M, and S in all patient groups.
444 **D.** Frequency of CD8 T cell responses to the tested viral components N, M, and S in all patient
445 groups. Bars represent the median value in each group. P values in the plots indicate the
446 significance by Mann-Whitney test.

447

448 **Figure 2. Memory phenotype characterization of SARS-CoV-2-specific T cell responses. A.**
449 A representative flow plot of the essential markers used for memory phenotyping. **B.** Memory
450 phenotypes of IFNγ+ CD4 T cells to SARS-CoV-2 N protein as an example in each patient
451 separated by patient group. **C.** Memory phenotypes of IFNγ+ CD8 T cells to SARS-CoV-2 N
452 protein in each patient separated by patient group.

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454 **Figure 3. Polyfunctionality, cytotoxicity, and activation of SARS-CoV-2-specific CD4 or CD8**
455 **T cells. A.** Polyfunctionality of CD4 T cell responses to SARS-CoV-2 N protein measured by IL2
456 and IFNγ. **B.** Cytotoxicity of IFNγ+ CD8 T cells to SARS-CoV-2 N protein measured by
457 expression of intracellular Granzyme B and membrane CD107a. **C.** Frequencies of CD69 and PD-
458 1 in SARS-CoV-2 N-specific IFNγ+ CD4 T cells in each patient group. Grey dots in the inpatient
459 group represent inpatients without oxygen supplementation. **D.** A representative flow plot of
460 cytotoxic CD8 T cells by the expression of Granzyme B and CD107a.
461
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560
561

Table 1. Demographic table of participants

| | Hospitalized (N=14) | Outpatient (N=15) | Total (N=29) |
|---|---|---|---|
| **Male** | 8 (57.1%) | 8 (53.3%) | 16 (55.2%) |
| **Race** | | | |
| Asian | 2 (14.3%) | 2 (13.3%) | 4 (13.8%) |
| Black | 8 (57.1%) | 1 (6.7%) | 9 (31.0%) |
| Hispanic | 1 (7.1%) | 5 (33.3%) | 6 (20.7%) |
| Other | 0 (0.0%) | 1 (6.7%) | 1 (3.4%) |
| White | 3 (21.4%) | 6 (40.0%) | 9 (31.0%) |
| **Age** | | | |
| Median (Q1, Q3) | 50.6 (45.3, 55.8) | 44.6 (39.7, 50.9) | 46.4 (40.1, 53.5) |
| Min - Max | 21.4 - 72.4 | 20.1 - 60.2 | 20.1 - 72.4 |
| **Age group** | | | |
| <18 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| 18-44 | 3 (21.4%) | 8 (53.3%) | 11 (37.9%) |
| 45-64 | 10 (71.4%) | 7 (46.7%) | 17 (58.6%) |
| 65+ | 1 (7.1%) | 0 (0.0%) | 1 (3.4%) |
| **Military affiliation** | | | |
| Active duty | 2 (14.3%) | 5 (33.3%) | 7 (24.1%) |
| Dependent | 6 (42.9%) | 6 (40.0%) | 12 (41.4%) |
| Retired military | 6 (42.9%) | 4 (26.7%) | 10 (34.5%) |
| **Any comorbidities** | 13 (92.9%) | 7 (46.7%) | 20 (69.0%) |
| **Multiple comorbidities** | 4 (28.6%) | 2 (13.3%) | 6 (20.7%) |
| **Charlson comorbidity index** | | | |
| Median (Q1, Q3) | 1.0 (0.0, 1.5) | 0.0 (0.0, 1.5) | 1.0 (0.0, 1.75) |
| Min - Max | 0 - 6 | 0 – 3 | 0 - 6 |

Table 2. Frequencies of T cell responses to endemic CoV and SARS-CoV-2 viral components

| | HKU-S | NL63-S | OC43-S | 229E-S | | SARS-CoV-2 N | SARS-CoV-2 M | SARS-CoV-2 E |
|---|---|---|---|---|---|---|---|---|
| Inpatient, O2 | 3 of 7 | 4 of 6 | 3 of 6 | 2 of 7 | | 7 of 11 | 9 of 11 | 0 of 11 |
| Inpatient, no O2 | 3 of 6 | 3 of 6 | 3 of 6 | 2 of 6 | | 3 of 3 | 3 of 3 | 2 of 3 |
| outpatient | 4 of 14 | 4 of 14 | 5 of 14 | 4 of 14 | | 6 of 15 | 7 of 15 | 1 of 15 |

Fig 1

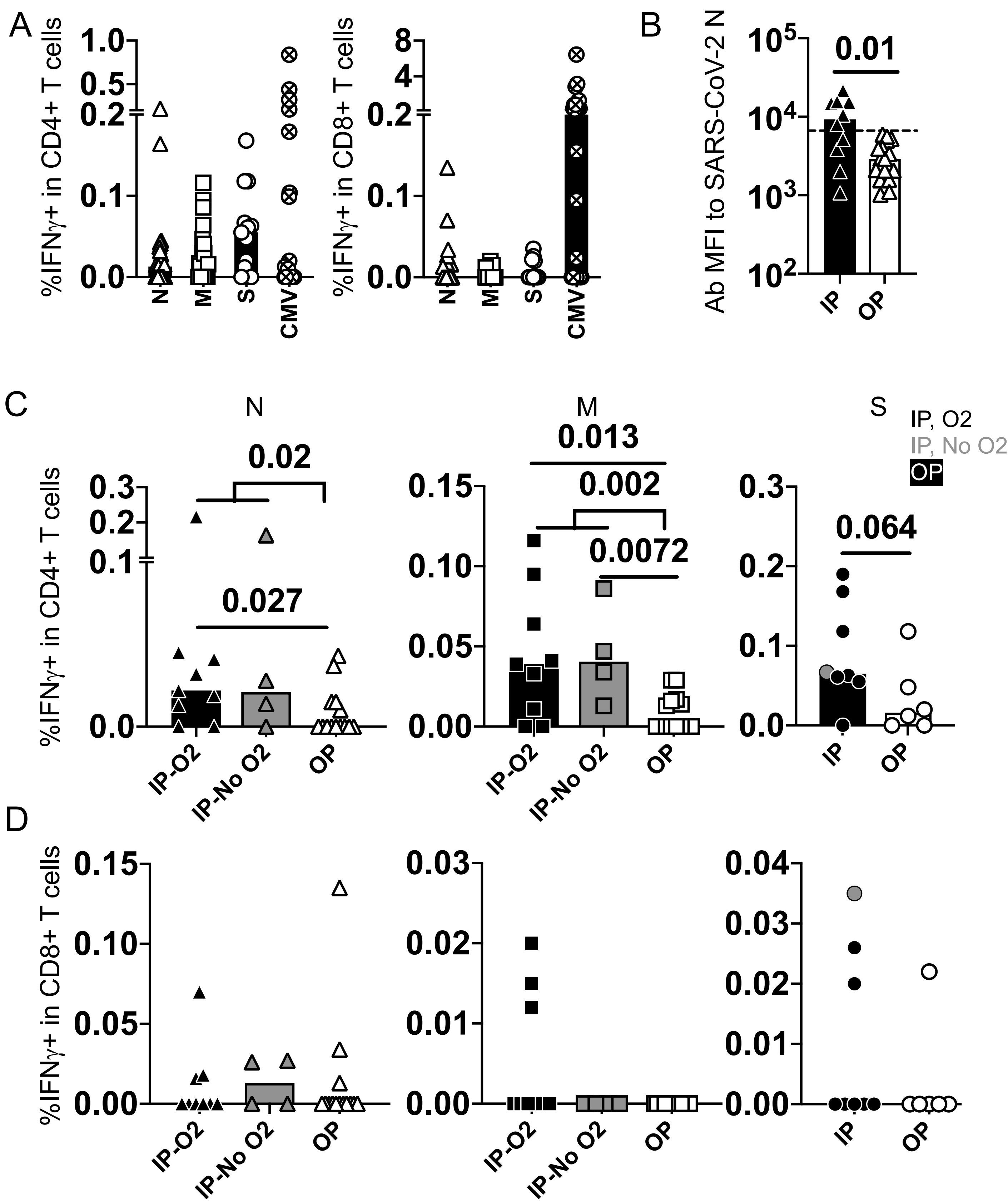
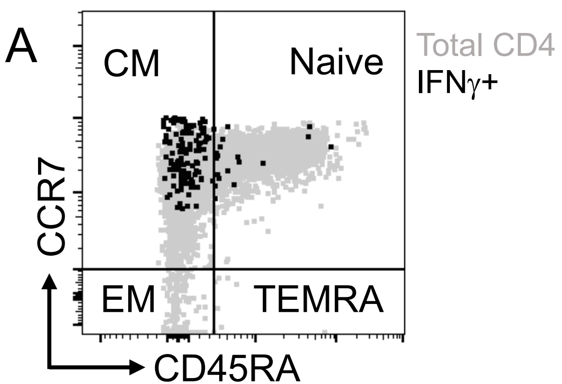
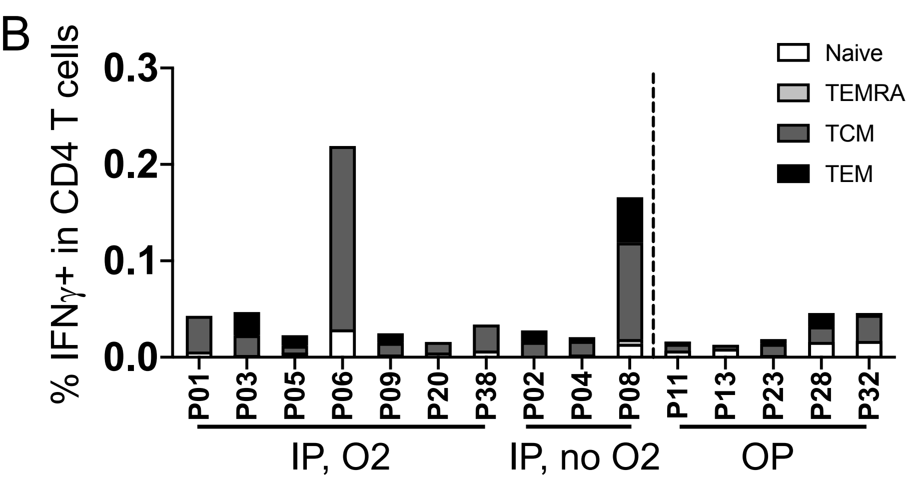


Fig 2



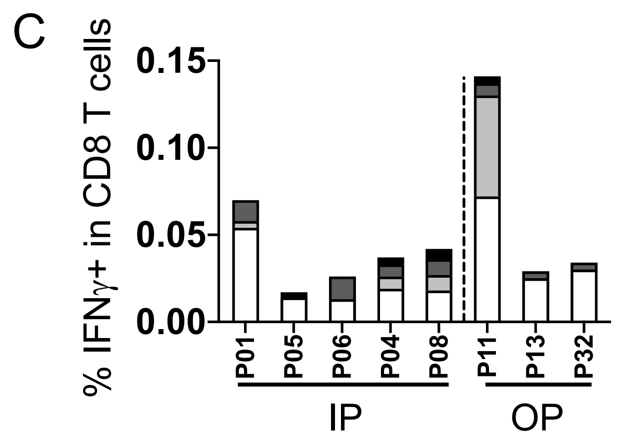



Fig 3